Biochemistry

SITE-DIRECTED MUTAGENESIS OF BOVINE DEOXYHYPUSINE SYNTHASE

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Hypusine (4-amino-2 (R)-hydroxylbutyl) lysine) synthesis in a mature eIF-5A is a unique two-step posttranslational modification involving two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DHH). Inhibitors of either the DS or DHH have been shown to exert antiproliferative and antiretroviral effects. A detailed knowledge of structure-function relationship of these enzymes will help in designing better inhibitors.

Previous work done by others in this laboratory have shown that bovine DHS possess considerably higher activity than the human protein in catalyzing the synthesis of deoxyhypusine. The amino acid sequence of bovine DHS shares 93% identity with the enzyme from humans, including 23 substitutions. In an effort to elucidate the underlying reason for the differences, in terms of specific amino acids, we are preparing amino acid substitution mutants F139L and L192M of bovine DHS. Each bovine DHS mutant DNA was prepared by site-directed mutagenesis using polymerase chain reaction primed with mutagenic oligonucleotides followed by overlap extension. The mutant DNA will be subcloned, expressed and characterized.

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